515. PROTEINASE SYSTEM OF A FUSARIUM CULTURE

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Although it is known that fungi grown on suitable substrates yield a wide variety of enzymes, relatively a few molds have been exploited as industrial sources of microbial enzymes. Amongst the cultures used, members of the genus *Aspergillus* have been extensively investigated for the production of various enzymes (Crewther & Lennox, 1953; Matsushira, 1958, 1959; Gillespie & Woods, 1953). Some species belonging to *Fusarium* are also known to produce pectic and cellulytic enzymes as well as proteinases (Togama, 1952; Sukhano & Podgainaja, 1951; Sampath Narayan & Shanmugasundaram, 1966). The present study relates to the partially purified proteinase of a *Fusarium* species isolated during the course of our search for micro-organisms capable of producing milk clotting enzymes for use in Cheese making.

EXPERIMENTAL

Production of Enzyme.—The culture (No. M-170) used in this study was isolated from soil and was maintained on potato–dextrose–agar. The spore suspension prepared in saline was used for inoculation into wheat bran medium consisting of 50 g wheat bran, 1·0 ml skim-milk and 50 ml water, pH 6·8. The medium was spread in thin layers in petridishees, sterilised at 15 psi for 30 minutes and cooled to room temperature. The enzyme formed after 4 days growth at 25° C was extracted with distilled water and the suspended particles filtered off.

Purification of the Enzyme.—The liquid containing the enzyme was precipitated with ammonium sulphate to 70 per cent saturation (at 4° C). The precipitate was collected by centrifugation and dried *in vacuo*. A 10 per cent solution of the enzyme in water was fractionated (at 4° C) which indicated that the fraction obtained between 0·2 and 0·4 saturation had maximum specific activity. This was dialysed for 3 hours at 4° C against 0·02 *M tris* buffer (pH 8·2). This fraction represented a purification of 270 fold for milk clotting activity and about 40-fold for proteolytic activity. Milk clotting and proteolytic activities were determined according to Srinivasan *et al.* (1964).

The heat stability of the partially purified proteolytic enzyme was determined by adjusting reaction mixture to pH 5·1, 6·0, 6·9, 8·2 and 9·0 and exposing to a temperature of 40°, 50° and 60° C for 15 minutes; portions were removed, cooled instantly and assayed for proteolytic activity using casein as substrate at 40° C.

RESULTS AND DISCUSSION

Effect of pH on Enzyme Activity.—Optimum pH of proteinase was determined by incubating enzyme of suitable dilution with Hammersten soluble casein as substrate at pH values varying from 5 to 10 employing acetate-phosphate-*tris*-HCl-carbonate buffers to cover the entire pH range. The reaction was carried out at 40° C for 20 minutes. A sharp optimum at about pH 8·5 was obtained with progressive decrease in the rate of hydrolysis of the substrate on either side of this value (Fig. 1).

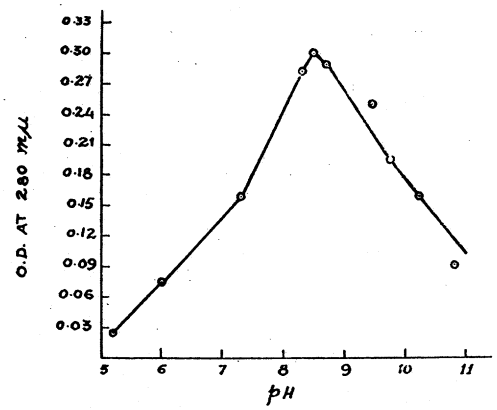

FIG. 1. Effect of pH on reaction rate

Effect of the Substrate Concentration.—It has been found (Table 1) that upto a substrate concentration of 5·0 mg/ml the reaction rate was proportional to the substrate concentration

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TABLE 1. *Casein Concentration and Enzyme Activity*

Casein concentration (mg)	Enzyme activity (units × 10^{-5})
5·0	12·21
7·5	15·00
10·0	19·80
20·0	21·35
30·0	21·45
40·0	20·00
50·0	18·65

0·5 ml to 2·5 ml of casein solution (2 per cent in *Tris* buffer 8·18, 0·2 M), 1·0 ml enzyme solution containing 130 μg protein, water to volume 4·0 ml.

and remained unaffected from 5 mg to 15 mg. At concentration between 15 to 25 mg, there was a decline in the reaction rate. The Michaelis-Menten constant for the reaction was calculated to be $0·5 \times 10^{-5}$M (Fig. 2) assuming the molecular weight of the casein as 75,000 (Buck *et al.*, 1962).

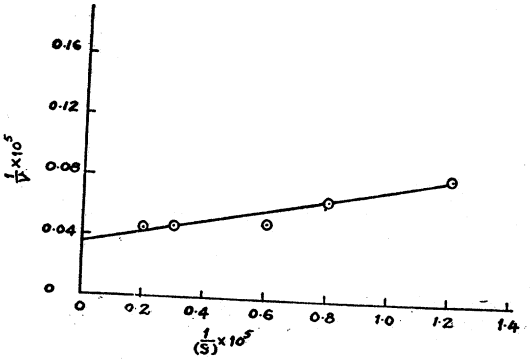

FIG. 2. Determination of km.

Effect of Enzyme Concentration.—In experiments on the effect of enzyme concentration on the rate of hydrolysis, enzyme concentration of 30–160 μg protein were employed keeping other conditions constant. It was found that the activity of the enzyme was linear upto 20 minutes, the experimental period (Fig. 3).

Heat Stability in Relation to pH and Temperature.—Results of trials on heat stability of casein digesting and milk clotting activities at combinations of pH and temperatures have been given in Table 2 and Table 3.

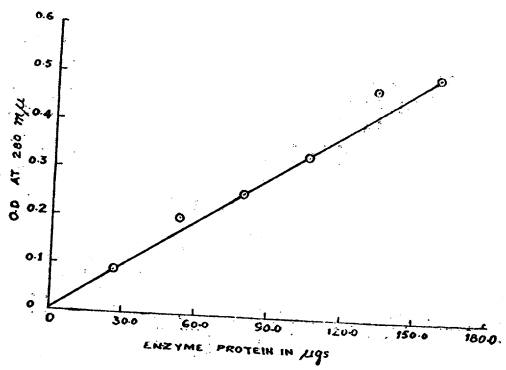

FIG. 3. Effect of enzyme concentration on reaction rate.

TABLE 2. *Degree of Inactivation of Casein Digesting Activity during Heat Treatment for 15 Minutes at Various pH*

pH	Percentage loss in activity at temperature °C.*		
	40	50	60
5·1	55·1	96·2	95·1
6·0	19·7	62·9	74·6
6·9	31·9	53·0	72·4
8·2	50·0	76·5	97·5
9·0	62·8	79·2	94·7

* Expressed as percentage activity at 20°C., enzyme used 90 μg./ml.

TABLE 3. *Heat Stability of Milk Clotting Activity for 15 Minutes*

Temperature °C.	Petcentage loss in activity at pH*	
	5·0	6·05
40	31·7	13·0
50	69·7	32·5
60	100·0	86·6

*Expressed as percentage activity at 20° C. pH 6·0. Enzyme used 90 μg./ml.

Maximum stability of casein digesting component of the enzyme preparation was observed at pH 6·0 at 40° 3. At 50 °C the loss in activity varied from 53 to 96 per cent. At 60° C the loss in activity was generally above 90 per cent at all pH values except at pH 6·0 and 6·9

where the loss was about 72 per cent. Data on milk clotting activity (Table 3) showed that the loss in activity at pH 5·0 was appreciably more than at pH 6·0 at the temperatures used. The losses in milk clotting activity were found to be considerably lower in comparison to losses in casein digesting activity at corresponding pH and temperatures (Compare Table 2). It may also be seen from the comparative data that while both types of activities were maximum at pH 6·0 (40° C) the losses in milk clotting activity at 50 °C and 60° C were considerably lower at pH 6·0 than at pH 5·0.

Time-Activity Relation—Fig. 4.

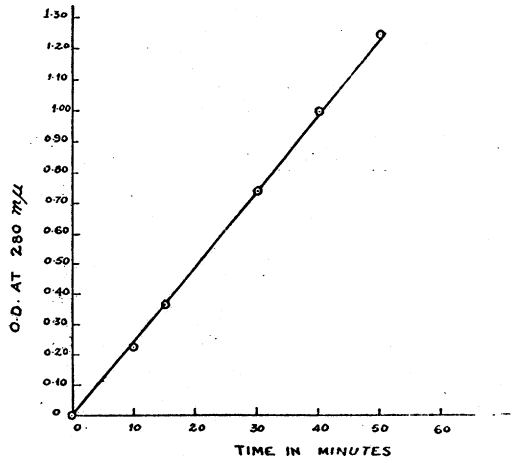

FIG. 4. Rate of hydrolysis of casein during first 60 minutes of reaction.

The data showed that the assay was confined to linear region.

Hydrolysis of Protein Substrates.—The proteinase system was tested for its ability to hydrolyse a few protein substrates of vegetable and animal origin. The substrates tested being gluten, peanut cake protein, blood albumin, egg albumin, casein soluble according to Hammersten and cow milk casein prepared according to the method of Dunn (1950). The results have been given in Table 4.

Maximum hydrolysis was found in case of casein. Blood albumin and peanut cake protein were hydrolysed appreciably while gluten and egg albumin showed very low hydrolysis, egg albumin being least hydrolysed of all the protein substrates examined.

TABLE 4. *Effect of Proteolytic Enzyme of* Fusarium *sp. on Hydrolysis of Different Substrates*

Protein	Hydrolysis/μg. enzyme protein/min. ($\times 10^{-4}$)
Cow casein ..	2·15
Gluten ..	0·18
Blood hæmoglobin ..	1·05
Peanut protein ..	0·85
Egg albumin ..	0·09

SUMMARY

Proteolytic and milk clotting enzymes have been purified 40-fold and 270-fold respectively from a culture belonging to *Fusarium* sp. The proteolytic enzyme had pH optimum at 8·5 and Michaelis–Menten constant according to the plot of Lineweaver and Burk was $0{\cdot}5 \times 10^{-5}$ M. Among the protein substrates casein was hydrolysed to maximum extent and egg albumin least.

Both enzymes exhibited maximum stability at pH 6·0 and temperature 40° C.

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